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Research Article

Differential Sensitivity of *Pisum sativum* L. Cultivars to Water-deficit Stress: Changes in Growth, Water Status, Chlorophyll Fluorescence and Gas Exchange Attributes

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Abstract

In recent years, drought has been a serious problem in Ethiopia and elsewhere which has adversely affected plant productivity. This study aimed to investigate the effects of water stress on growth, biomass and foliar characteristics in three cultivars (Brukitu, Tegegnech and Adi) of *Pisum sativum*. The control plant pots were uniformly irrigated at 3 day intervals to maintain 100% field capacity. Water-stress conditions were imposed by subjecting plants to a gradual decrease of soil water availability such as watering at 6 day intervals (slight-stress condition), 9 day intervals (mild-stress condition) and 12 day intervals (severe-stress condition). Results revealed significant differences among the cultivars, water-stress treatments and their interaction, indicating the cultivars variability and differential response to water stress. Water stress adversely affected growth, biomass production, leaf water status and other leaf characteristics such as pigment concentration (chlorophyll a, b and total chlorophyll), maximum quantum yield of photosystem II (PS II) (Fv/Fm), net photosynthetic rate, stomatal conductance and transpiration rate in all cultivars, as stress level was increased in comparison to control plants. The relatively less decline in the studied parameters of Tegegnech exhibited a reasonable tolerance ability of this cultivar, whereas Brukitu and Adi proved to be sensitive to water-deficit condition.

Key words: Biomass, pigment, chlorophyll fluorescence, pea, photosynthetic rate, relative water content, drought stress

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Abiotic stresses such as drought and salinity are the major environmental constraints, which make taking water from the soil more difficult for the plant, due to an increase in the osmotic pressure of the soil solution as compared to that of roots. Currently, availability of a sufficient amount of water for irrigation is becoming a serious problem; therefore, many efforts have been concerted on the development and screening of plant varieties/cultivars demanding less water, which can complete their life cycle in drought-prone areas such as in Ethiopia and elsewhere (Ezra, 2001; Kang *et al*., 2009; Lobell and Gourdji, 2012; Elliott *et al*., 2014).

Plants under water-stress condition responded by a number of physiological mechanisms at the molecular, cellular, tissue, morphological and whole-plant levels (Anjum *et al*., 2008; Husen, 2010; Prasch and Sonnewald, 2013; Tripathi *et al*., 2014; Husen *et al*., 2014; Chaumont and Tyerman, 2014; De Ollas *et al*., 2015). These responses vary with the species and genotype/cultivars, the length and severity of water stress and the developmental stage. Reduction in soil water availability leads to a low plant water potential and a consequent loss of turgidity and inhibition of cell elongation in leaves. The effect of water stress on the net photosynthesis has been traditionally studied in terms of 'Stomatal' and 'Non stomatal' limitations, the former resulting from the resistance of CO_2 diffusion to intercellular leaf space and the latter being often completely assumed as a metabolic constraint (Chaves *et al*., 2009). Plants tend to avoid excessive transpiration by closing the stomata (Flexas *et al*., 2004). This reduces the gaseous exchange between leaf and the atmosphere, leading to a low intercellular CO_2 concentration (Flexas *et al*., 2004), reduced diffusion of CO_2 to chloroplasts and a limited net CO_2-assimilation rate (Chaves *et al*., 2002) with ensuing negative feedback in photochemical efficiency (Ribeiro *et al*., 2008). Water-stress condition also affects leaf-area expansion (Husen *et al*., 2014), absorption of photosynthetically active radiation and the leaf efficiency to carry out carbon fixation (Flexas *et al*., 2004). However, plants exhibit adaptive cellular responses like up-regulation of oxidative-stress protectors and accumulation of protective solutes, besides leaf area adjustments that reduce water loss by transpiration (Anjum *et al*., 2008, 2012).

Efficiency of photosystem II (PS II), measured as chlorophyll fluorescence (maximum quantum yield Fv/Fm), has been used extensively as a diagnostic tool in studies of the abiotic stresses (Baker and Rosenqvist, 2004; Getnet *et al*., 2015; Husen *et al*., 2014, 2016), genotypic variation (Husen, 2010), altitudinal variation (Husen *et al*., 2004a) and species-specific diurnal changes (Husen *et al*., 2004b) on the PS II electron transfer process (Baker, 2008), thus acting as an indicator of seedling-stock quality (Husen, 2009; Hanachi *et al*., 2014; Getnet *et al*., 2015). Reduction in the quantum yield of photosystem is influenced not only by light intensity but also by the superimposition of other environmental stresses such as high temperature, salinity, water availability or CO_2 supply (Souza *et al*., 2004; Ribeiro *et al*., 2008; Husen *et al*., 2014, 2016). Water stress inhibits photosynthetic activity in tissues due to imbalance between light capture and its utilization (Foyer and Noctor, 2000). Under these conditions, plants develop several strategies to avoid photoinhibitory processes, e.g., mechanisms to prevent or dissipate excessive light absorption or mechanisms to consume the reducing power generated by PS II (Demmig-Adams and Adams, 1992).

Ethiopia is one of the richest centers in the world in terms of crop diversity (Husen *et al*., 2012). *Pisum sativum* L. is widely cultivated at the altitudes between 1800 and 3000 m above mean sea level with annual average rainfall of 700-900 mm in the different regions of Amhara, Oromia, Tigray and Southern Ethiopia (EEPA., 2004). It is the second most important pulse crop in the country after faba bean in terms of both area coverage and production. According to the CSA (2008), field pea covers over 254,000 ha with total production of 230,000 t that accounts for 17% of the total grain legume production. *Pisum sativum* is most important food and feed crop with high contents of protein and vitamins. Consequently, it is an inexpensive source of protein and cooked as sauces to supplement carbohydrate rich food for many people (EEPA., 2004). Moreover, pulses also offer natural soil maintenance benefits through nitrogen-fixing, which improves yields of cereals through crop rotation and can also result in savings for smallholder farmers from less fertilizer use (Chilot *et al*., 2010). The wide range of variation that exists among different pulses and their cultivars may be utilized gainfully for identifying and developing the water-resistant/tolerant candidates. In Ethiopia, during last 30 years, four major drought periods and associated famines of varying degrees of severity have been recorded (Henricksen and Durkin, 1985). In recent years, the severity of this drought increased more due to increasingly erratic rainfall patterns as exacerbated by the ocean-warming trend El Niño (EHCT., 2015). While growing in the drought-prone parts of Ethiopia (Ezra, 2001), *P. sativum* has to suffer from water stress. Therefore, the present study aims to determine water stress effects on three cultivars of *P. sativum* for their growth, leaf characteristics, water status and physiological activities, in view of that they could differ in terms of tolerance capacity against stress.

MATERIALS AND METHODS

Experimental site: The experiments were conducted in the Botanical Science Research Laboratory (Department of Biology) at the Tewodros campus of the University of Gondar located at 12°35' 14.19" N, 37°26' 29.53" E at 2143 m above mean sea level. The annual average of the maximum and minimum daily temperature at Gondar lies around 27 and 16°C, respectively. March-May is the hottest period, with average maximum temperature 29°C. Average precipitation in Gondar is about 1161 mm per annum, which means a monthly precipitation of 96.75 mm. The annual average of daily Relative Humidity (RH) is about 56%, the lowest (40%) occurring in January and February and the highest (79%) in July. During the entire experimental period, RH was 50%, the maximum and the minimum daily temperature was recorded as 29±1 and 18±1°C, respectively and no rainfall took place.

Plant material, experimental design and water stress treatments: Seeds of Brukitu, Tegegnech and Adi three cultivars of *Pisum sativum* L., were obtained from Gondar Agricultural Research Centre and surface sterilized with 80% ethyl alcohol for 15 min, followed by repeated washings with distilled water. The clean seeds of each cultivar were then sown in separate plastic trays containing 75% soil and 25% farmyard manure (FYM) and being watered regularly. After 2 weeks of germination, uniform seedlings chosen from each cultivar were transferred separately to plastic pots (8 cm width×16 cm height) filled with 1.5 kg soil and 500 g FYM in 3:1 ratio, sown at a depth of 2 cm and irrigated at 100% Field Capacity (FC) daily with tap water for the next 2 weeks supposedly a period of plant acclimatization. To estimate the FC, the soil was sun dried for 10 days and its waterholding capacity was measured in the form of amount of water (mL) required to saturate 1 kg of dried soil, which is then used as percent FC. The soil in pots was sandy loam (62.56% sand, 14.88% clay and 22.56% silt), with pH 7.23 and EC 0.69 ms cm^{-1}. Pots were arranged in a Completely Randomized Design (CRD) and water stress treatments were applied in 5 replicates. The control plant pots (T1) were uniformly irrigated with tap water (400 mL kg^{-1} soil) at 3 day intervals to maintain almost 100% FC. Further water stress was imposed by subjecting plants to a gradual decrease of soil water availability such as watering at 6 day intervals (T2: Slight-water stress), 9 day intervals (T3: Mild-water stress) and 12 day intervals (T4: Severe-water stress). Water stress condition was maintained 30-70 days. Seventy days after sowing, the control and treated plants of the cultivars were harvested and analyzed for growth and physiological parameters.

Plant growth: Seventy days after sowing, the growth parameters of three pea cultivars were measured in the control and water stress conditions. The seedlings were gently uprooted for recording the plant length (cm), number of branches and the size, area and number of opened leaves. Ground-line basal diameter (mm) of stem was measured with an electronic digital caliper. The width (mm), length (mm) and area (mm^2) of leaves were measured with the help of a leaf area meter (AM 300, ADC Bio Scientific Limited, U.K.). Five replications were used for determining each parameter.

Biometric studies: Pea cultivars from each treatment were harvested and divided in roots, stems and leaves. For each sample, five replications were used. Roots were washed carefully with tap water and excess water was removed using blotting paper. All the plant parts were oven-dried separately at 85°C for 2 days when the weight became constant. Root Biomass (RB), Stem Biomass (SB) and Leaf Biomass (LB) were then determined by using an electronic digital balance (Citizen Scale, CY510, Poland). From these data, the following biometric traits (g) were calculated:

- Total Biomass (TB) = RB+LB+SB
- Root to shoot ratio (R/S) = RB/(SB+LB)
- Root dry mass ratio (RMR) = RB/TB
- Stem dry mass ratio (SMR) = SB/TB
- Leaf dry mass ratio (LMR) = LB/TB

Chlorophyll analysis: Chlorophyll content was analyzed in randomly collected leaves of each cultivar, using three replications per cultivar. Approximately, 100 mg of fresh leaves was used and chlorophyll pigments were extracted with 80% acetone. The absorbance was measured at 645 and 663 nm, using a T60 UV/Vis spectrophotometer (PG Instruments Limited, England). Thereafter, chlorophyll a, chlorophyll b and total chlorophyll were calculated according to Arnon (1949) and expressed in μg mL^{-1}.

Relative water content: Water status of leaf was determined in fully developed leaves of the control and water-stressed plants by measuring the Relative Water Content (RWC). Three replications per cultivars were used. Leaf samples were weighed immediately after harvesting, to obtain fresh weight and then kept overnight in distilled water at 5°C in the dark, before obtaining their Turgid Weight (TW). The material was then oven-dried at 85°C for 48 h and Dry Weight (DW) obtained. The relative water content was calculated as:

$$RWC = \{(FW-DW) \div (TW-DW)\} \times 100$$

Chlorophyll fluorescence: Chlorophyll fluorescence of leaves was recorded in the forenoon (10-11 AM) for each treatment with the help of a portable Multi-Mode OS5p Chlorophyll Fluorimeter (Opti-Sciences, Inc., USA). Prior to fluorescence measurements, the upper surface of the leaf was pre-darkened with leaf clips for 30 min to ensure complete relaxation of all reaction centres. The basal non-variable chlorophyll fluorescence (Fo), maximal fluorescence induction (Fm) and variable fluorescence (Fv) were determined. The maximum quantum yield of PS II (Fv/Fm) was estimated by the ratio Fv/Fm = (Fm-Fo)/Fm (Genty *et al*., 1989).

Foliar gas exchange: Leaf gas exchange was measured between 10 to 11 AM for each treatment. Stomatal conductance (gs), net photosynthetic rate (Pn) and transpiration rate (E) were measured using a portable leaf gas exchange system (ADC BioScientific Limited, U.K.) on fully expanded attached leaves. The equipment was used with the following specifications/adjustments: Leaf surface area 6.25 cm^2, ambient CO_2 concentration (C_{ref}) 371 µmol moL^{-1}, temperature of leaf chamber (Tch) 25-28°C, molar air flow per mater square of leaf surface (Us) 296 mol m^{-2} sec^{-1}, leaf chamber volume gas flow rate (v) 400 mL m^{-1}, ambient pressure (P) 97.95 kPa, PAR (Q_{leaf}) at leaf surface up to 770 µmol m^{-2} sec^{-1}.

Statistical analysis: Statistical analysis of data was performed with version 16.0 Statistical Package for Social Sciences (SPSS) software package (SPSS Inc., Illinois, USA). The data was subjected to a two-way (Cultivars: Brukitu, Tegegnech and Adi×Water-stress treatments) analysis of variance (ANOVA) to determine the significant difference among the treatments and cultivars. Differences among the mean values were assessed by Least Significant Differences (LSD) at significance level $p<0.05$ (values marked with the same letters within a row or column are not significantly different at $p>0.05$ level).

RESULTS

Plant growth: Water-stress treatments significantly affected all the growth and leaf characteristics parameters except number of branches. The effect on the cultivars was significant only for number of leaves. However, the interactive effect of water-stress treatmentsxcultivars was significant for all the parameters studied (Table 1). The maximum number of leaves was found in the cultivar Tegegnech (Table 2). As the water-stress level increased, the growth parameters declined significantly (Table 2). The control plants were taller in comparison to the water-stress condition of each cultivar. In comparison to control, plant height was reduced by 57.09, 53.54 and 49.03% (Fig. 1a), whereas the basal diameter increment declined by 55.56, 57.54 and 55.56% (Fig. 1b) in cultivars Brukitu, Tegegnech and Adi, respectively, at the severe-water stress condition. The numbers of leaves were also reduced at severe-water stress condition by 28.31, 30.10

Table 1: Analysis of variance results on the effect of cultivars, water-stress treatments and their combination for growth, biometric traits, chlorophyll contents and physiological parameters

	Cultivars			Water-stress treatments			Cultivars × water-stress treatments		
Parameters	MSS	p<0.05	p<0.01	MSS	p<0.05	p<0.01	MSS	p<0.05	p<0.01
Height (cm)	18.48	-	-	3892.54	-	**	243.69	*	-
Stem basal diameter (mm)	0.84	-	-	10.92	*	-	0.08	*	-
Number of leaf	486.56	*	-	2246.78	*	-	222.55	*	-
Number of branch	8.23	-	-	202.35	-	-	10.87	*	-
Leaf area (mm^2)	2729154.79	-	-	2.68	-	**	6198559.33	-	**
Leaf width (mm)	34.25	-	-	35.66	*	-	35.69	*	-
Leaf length (mm)	16.24	-	-	1198.88	*	-	214.84	*	-
Total biomass	1.90	*	-	12.59	*	-	0.20	-	**
Root/shoot ratio	0.20	-	-	0.02	*	-	0.04	*	-
Root dry mass ratio	0.13	-	-	0.01	*	-	0.02	*	-
Stem dry mass ratio	0.17	*	-	0.03	-	**	0.01	*	-
Leaf dry mass ratio	0.03	*	-	0.02	-	**	0.02	*	-
Chlorophyll a	14.87	*	-	44.79	-	**	4.20	-	**
Chlorophyll b	61.42	*	-	133.57	*	-	14.95	*	-
Total chlorophyll	137.78	*	-	332.09	*	-	34.59	-	**
Relative water content (%)	330.14	*	-	761.56	-	**	67.62	-	**
Chlorophyll fluorescence	0.034	*	-	0.072	*	-	0.023	*	-
Photosynthetic rate (µmol m^{-2} sec^{-1})	0.709	*	-	11.88	-	**	0.435	*	-
Stomata conductance (mol m^{-2} sec^{-1})	0.006	-	-	0.009	*	-	0.007	*	-
Transpiration rate (m mol m^{-2} sec^{-1})	0.020	*	-	2.88	*	-	0.080	*	-

MSS: Mean square value, *Significant at $p<0.05$ and **Significance at $p<0.01$

Table 2: Effects of water-stress treatments on different growth and leaf characteristic features in selected cultivars of *Pisum sativum*

		Water-stress treatments			
Parameters	Cultivars	T1	T2	T3	T4
Height (cm)	Brukitu	65.00±3.18^{a}	52.33±3.18^{c}	42.11±3.28^{e}	27.89±1.68^{g}
	Tegegnech	68.89±3.02^{a}	56.28±2.17^{b}	45.85±3.21^{d}	32.00±2.04^{f}
	Adi	62.78±2.79^{a}	49.86±3.06^{c}	40.11±2.77^{e}	26.00±1.95^{g}
Stem basal diameter (mm)	Brukitu	2.43±0.71^{a}	1.72±0.32^{b}	1.28±0.31^{d}	1.08±0.12^{e}
	Tegegnech	2.85±0.74^{a}	2.03±0.28^{b}	1.49±0.36^{c}	1.21±0.38^{d}
	Adi	2.34±0.67^{a}	1.82±0.29^{b}	1.37±0.30^{d}	1.04±0.10^{e}
Number of leaf	Brukitu	75.00±3.17^{b}	64.00±2.21^{c}	59.22±3.74^{c}	53.77±2.65^{d}
	Tegegnech	85.88±4.53^{a}	64.88±2.81^{c}	60.66±4.87^{c}	54.88±2.73^{d}
	Adi	72.86±3.87^{b}	64.22±2.17^{c}	54.88±3.11^{d}	50.06±2.17^{e}
Number of branch	Brukitu	16.66±2.87^{a}	13.66±3.62^{a}	12.42±2.71^{a}	11.55±1.05^{b}
	Tegegnech	18.77±2.93^{a}	14.88±2.85^{a}	13.11±1.78^{a}	11.77±2.01^{ab}
	Adi	15.88±2.73^{a}	13.35±2.94^{a}	12.00±1.62^{b}	11.00±1.62^{b}
Leaf area (mm^{2})	Brukitu	13573.37±465.93^{a}	13186.37±426.01^{b}	12077.38±412.16^{b}	10054.37±454.05^{d}
	Tegegnech	13782.23±547.48^{a}	13373.25±551.48^{a}	12445.97±379.68^{b}	11611.38±479.82^{c}
	Adi	12768.17±564.25^{a}	11726.72±462.07^{c}	11448.63±473.73^{c}	9565.78±586.39^{d}
Leaf width (mm)	Brukitu	105.10±3.59^{a}	97.56±3.64^{b}	85.23±4.96^{c}	76.16±4.83^{d}
	Tegegnech	108.28±3.28^{a}	105.34±3.84^{a}	98.75±3.95^{b}	80.20±3.43^{c}
	Adi	104.10±3.87^{a}	94.36±3.02^{b}	80.47±3.94^{c}	72.94±4.28^{d}
Leaf length (mm)	Brukitu	145.90±4.74^{a}	129.77±5.53^{b}	127.83±4.74^{b}	107.20±4.63^{d}
	Tegegnech	147.43±4.88^{a}	131.63±4.86^{b}	130.63±4.81^{b}	115.50±5.52^{c}
	Adi	145.97±4.95^{a}	128.77±5.73^{b}	127.07±5.42^{b}	106.93±5.07^{d}

T1: Control plant and uniformly irrigated with tap water (400 mL kg^{-1} soil) at 3 day intervals to maintain 100% FC, T2: Irrigated at 6 day intervals (slight-water stress), T3: Irrigated 9 day intervals (mild-water stress) and T4: Irrigated at 12 day intervals (severe-water stress). Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

and 31.29% for the respective three cultivars (Fig. 1c). The number of branches was decreased by 30.67, 37.29 and 30.73% at the severe-water stress level in Brukitu, Tegegnech and Adi, respectively (Fig. 1d). Leaf area expansion was reduced, with reference to the control, by 25.93, 15.75 and 25.08% (Fig. 1e), leaf width by 27.53, 25.93 and 29.92% (Fig. 1f) while the leaf length by 26.52, 21.65 and 26.74% in cultivar Brukitu, Tegegnech and Adi, respectively, (Fig. 1g). Cultivar Tegegnech was less affected under water stress in terms of the studied growth parameters (Fig. 1a-g).

Plant biometry: Analysis of variance has exhibited that water-stress treatments significantly affected the plant biometry (Table 1). Effect of cultivars was significant only for Total Biomass (TB), stem dry mass ratio (SMR) and leaf dry mass ratio (LMR). However, the interactive effect of water-stress treatments×cultivars was significant for all the studied parameters (Table 1). Cultivar Tegegnech revealed significantly higher TB, SMR and LMR in comparison to cultivar Brukitu and Adi. The TB, root-to-shoot ratio (R/S), root dry mass ratio (RMR), SMR and LMR was reduced as the stress level was increased from slight to severe-water stress condition. Control plants showed the higher TB, R/S, RMR, SMR and LMR in comparison to the stressed plants. Of the various stress levels, severe-water stress condition was the most effective in terms of reduction of plant biometry for each cultivar. In comparison to control, the reduction in TB at severe-water stress condition was up to 68.20, 65.00 and 70.00% in Brukitu, Tegegnech and Adi, respectively (Fig. 2a). At severe-water stress condition, the R/S was reduced by 39.12, 15.20 and 40.59%, RMR by 69.78, 41.30 and 71.27%, SMR by 42.77, 45.01 and 39.21% and LMR by 40.40, 35.49 and 42.42% in cultivar Brukitu, Tegegnech and Adi, respectively as compared with the control plant pots that were uniformly irrigated with 100% FC (Fig. 2b-e). Cultivar Tegegnech was able to maintain less percent variation and recorded the highest mean values for studied plant biometry at all water-stress condition (Table 3 and Fig. 2a-e).

Photosynthetic pigments: Analysis of variance exhibits that water-stress treatments, cultivars and cultivars×water-stress treatments interaction significantly affected chlorophyll a, chlorophyll b and total chlorophyll contents (Table 1). Compared with the control plants, the chlorophyll contents were significantly lower in water-stress condition (Table 4). Cultivar Tegegnech possessed the highest chlorophyll a, chlorophyll b and total chlorophyll contents among the three cultivars (Table 4). The contents of chlorophyll a, chlorophyll b and total chlorophyll were consistently reduced in all the three cultivars with increase in the water-stress levels. In comparison to the control plants, at severe-water stress condition, chlorophyll a declined by 47.96, 44.75 and 60.18%, chlorophyll b by 40.79, 40.99 and 44.24% and total chlorophyll

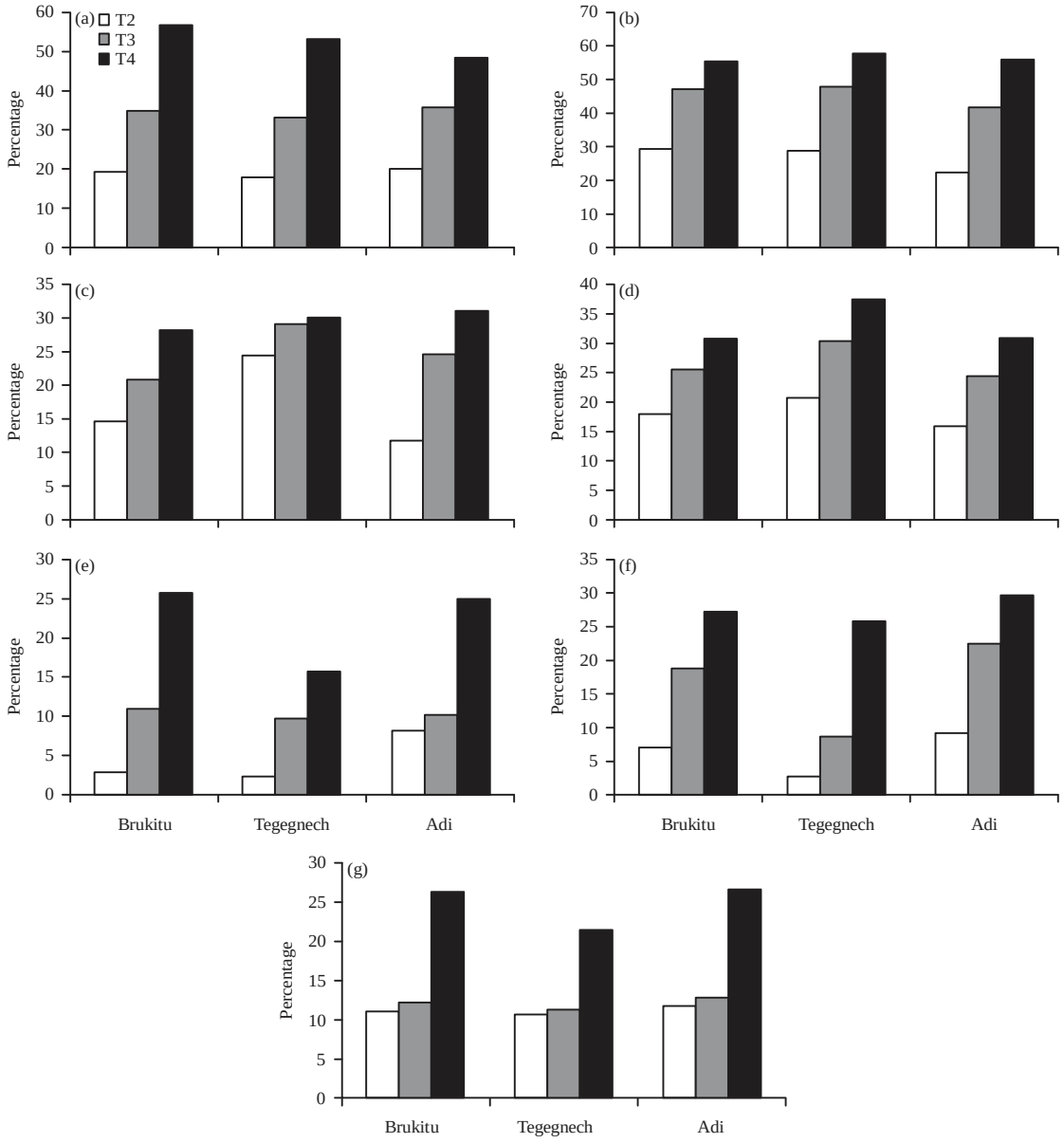

Fig. 1(a-g): Percent variation from the control, as observed under slight-water stress (T2), mild-water stress (T3) and severe-water stress (T4) conditions for, (a) Plant height, (b) Stem basal diameter, (c) Number of leaves, (d) Number of branches, (e) Leaf area, (f) Leaf width and (g) Leaf length in Brukitu, Tegegnech and Adi cultivars of *Pisum sativum*

by 45.18, 43.34 and 54.46% in cultivar Brukitu, Tegegnech and Adi, respectively (Fig. 3a-c). Accordingly, in terms of percent variation and the mean data value, cv Tegegnech retained the maximum chlorophyll a, chlorophyll b and total chlorophyll content among the cultivars (Table 4 and Fig. 3a-c).

Physiological attributes: Analysis of variance demonstrates that the water-stress treatments significantly affected the Relative Water Content (RWC), chlorophyll fluorescence (Fv/Fm), stomatal conductance (gs), net photosynthetic rate (Pn) and transpiration rate (E). The effect on the cultivars was significant for these physiological attributes, except for gs. However, cultivars×water-stress-treatments interaction was significant for all studied physiological attributes. Compared with the control, RWC was decreased under water-stressed plants; this was more as the stress level was increased from slight to severe-water stress condition (Table 5). At severe-water stress condition, the reduction was 31.71, 34.16 and 37.25% in Brukitu, Tegegnech and Adi, respectively (Fig. 4). Accordingly, in terms of percent variation and the

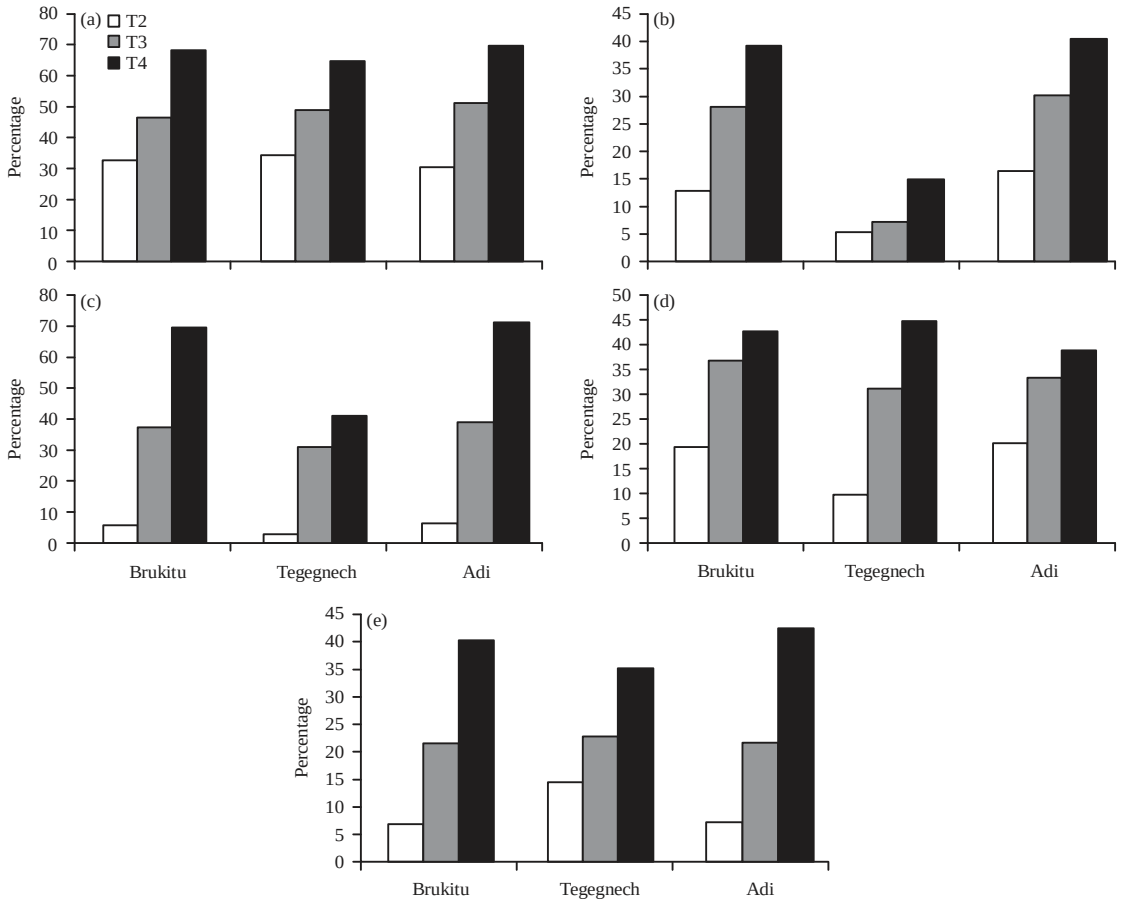

Fig. 2(a-e): Percent variation from the control, as observed under slight-water stress (T2), mild-water stress (T3) and severe-water stress (T4) conditions for, (a) Total biomass, (b) Root/shoot ratio, (c) Root dry mass ratio, (d) Stem dry mass ratio and (e) Leaf dry mass ratio in Brukitu, Tegegnech and Adi cultivars of *Pisum sativum*

Table 3: Effects of water-stress treatments on the biometry in selected cultivars of *Pisum sativum*

		Water- stress treatments			
Parameters	Cultivars (g)	T1	T2	T3	T4
Total biomass	Brukitu	2.170±0.19^{b}	1.450±0.21^{c}	1.150±0.20^{d}	0.690±0.27^{e}
	Tegegnech	2.720±0.25^{a}	1.780±0.19^{b}	1.390±0.22^{c}	0.952±0.31^{d}
	Adi	1.940±0.26^{b}	1.340±0.24^{c}	0.948±0.27^{d}	0.582±0.32^{e}
Root/shoot ratio	Brukitu	0.202±0.05^{a}	0.176±0.04^{c}	0.145±0.06^{d}	0.123±0.05^{f}
	Tegegnech	0.204±0.05^{a}	0.193±0.05^{b}	0.189±0.05^{b}	0.173±0.06^{e}
	Adi	0.202±0.06^{a}	0.169±0.06^{c}	0.141±0.05^{d}	0.120±0.05^{f}
Root dry mass ratio	Brukitu	0.182±0.03^{a}	0.171±0.03^{b}	0.114±0.04^{d}	0.055±0.03^{e}
	Tegegnech	0.184±0.04^{a}	0.179±0.04^{a}	0.126±0.03^{c}	0.108±0.04^{d}
	Adi	0.181±0.04^{a}	0.170±0.04^{b}	0.110±0.04^{d}	0.052±0.03^{e}
Stem dry mass ratio	Brukitu	0.484±0.05^{b}	0.390±0.06^{c}	0.305±0.05^{d}	0.277±0.031^{e}
	Tegegnech	0.551±0.04^{a}	0.496±0.05^{a}	0.338±0.03^{b}	0.303±0.03^{d}
	Adi	0.454±0.06^{b}	0.362±0.06^{c}	0.301±0.05^{d}	0.276±0.030^{e}
Leaf dry mass ratio	Brukitu	0.401±0.07^{b}	0.374±0.05^{c}	0.315±0.07^{d}	0.239±0.08^{e}
	Tegegnech	0.462±0.06^{a}	0.396±0.07^{b}	0.356±0.06^{c}	0.298±0.08^{d}
	Adi	0.396±0.06^{b}	0.368±0.08^{c}	0.310±0.08^{d}	0.228±0.07^{e}

T1: Control plant and uniformly irrigated with tap water (400 mL kg^{-1} soil) at 3 day intervals to maintain 100% FC, T2: Irrigated at 6 day intervals (slight-water stress), T3: Irrigated 9 day intervals (mild-water stress) and T4: Irrigated at 12 day intervals (severe-water stress). Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

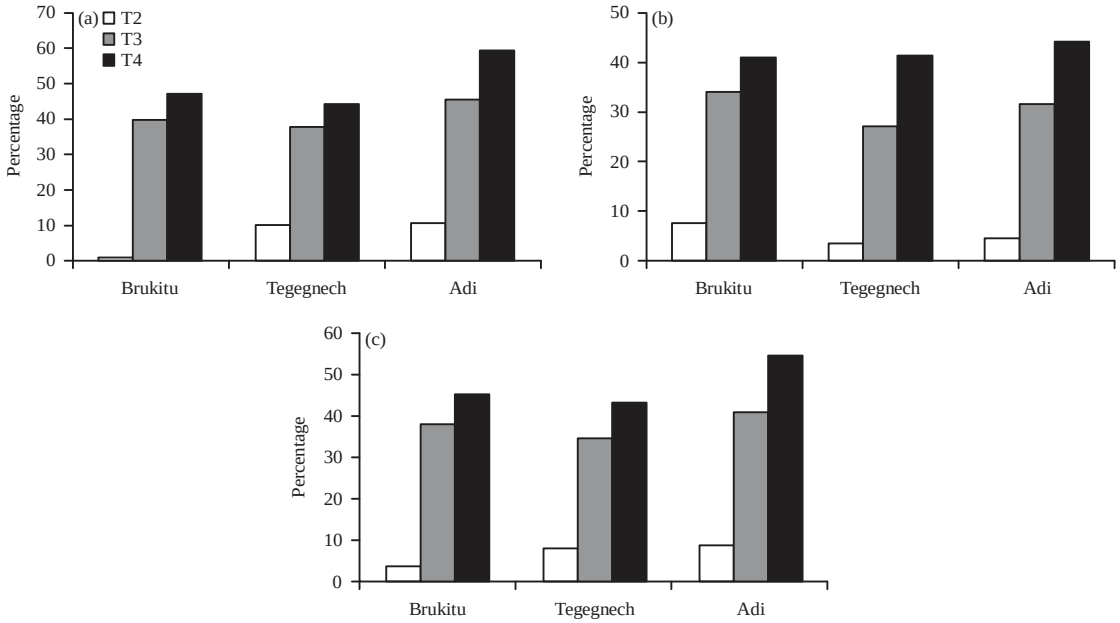

Fig. 3(a-c): Percent variation from the control, as observed under slight-water stress (T2), mild-water stress (T3) and severe-water stress (T4) conditions for, (a) Chlorophyll a, (b) Chlorophyll b and (c) Total chlorophyll in Brukitu, Tegegnech and Adi cultivars of *Pisum sativum*

Table 4: Effects of water-stress treatments on the photosynthetic pigments in selected cultivars of *Pisum sativum*

		Water-stress treatments			
Photosynthetic pigments (μg mL^{-1})	Cultivars	T1	T2	T3	T4
Chlorophyll a	Brukitu	7.63±0.84^{b}	7.55±0.42^{b}	4.55±0.63^{e}	3.97±0.75^{f}
	Tegegnech	8.85±0.63^{a}	7.90±0.63^{b}	5.43±0.72^{d}	4.89±0.74^{e}
	Adi	7.91±0.76^{b}	7.03±0.46^{c}	4.28±0.65^{e}	3.15±0.56^{f}
Chlorophyll b	Brukitu	4.83±0.62^{b}	4.48±0.53^{b}	3.19±0.52^{c}	2.86±0.51^{d}
	Tegegnech	5.27±0.54^{a}	5.10±0.74^{a}	3.85±0.75^{b}	3.11±0.68^{c}
	Adi	4.43±0.67^{b}	4.23±0.64^{b}	3.03±0.69^{c}	2.47±0.64^{d}
Total chlorophyll	Brukitu	12.46±0.63^{b}	12.03±0.70^{b}	7.74±0.64^{d}	6.83±0.52^{e}
	Tegegnech	14.12±0.74^{a}	13.00±0.68^{ab}	9.28±0.61^{c}	8.00±0.66^{d}
	Adi	12.34±0.72^{b}	11.26±0.74^{b}	7.31±0.72^{d}	5.62±0.72^{e}

T1: Control plant and uniformly irrigated with tap water (400 mL kg^{-1} soil) at 3 day intervals to maintain 100% FC, T2: Irrigated at 6 day intervals (slight-water stress), T3: Irrigated 9 day intervals (mild-water stress) and T4: Irrigated at 12 day intervals (severe-water stress). Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

mean data value, cv Tegegnech had the maximum RWC among the cultivars examined (Table 5). It tried to maintain RWC at the slight-stress condition. In addition, Tegegnech statistically exhibited a similar kind of response at severe water stress condition as Brukitu and Adi gave at mild- water stress condition. A significant decrease in Fv/Fm, gs, Pn and E was measured, especially at severe-water stress condition. The Fv/Fm value was greater in the control than in the treated plants. However, it was a bit insensitive statistically in cultivar Tegegnech (Table 5). At severe-water stress condition, it varied from the control by 10.94, 6.15 and 12.11% in Brukitu, Tegegnech and Adi, respectively (Fig. 5a). For Pn, all the cultivars exhibited significant variation from the control, cultivar Tegegnech being superior to the others. With increase in water stress level, Pn was reduced significantly. Compared with the control, the reduction in Pn went up to 52.69, 50.60 and 54.98% at severe-water stress condition, in Brukitu, Tegegnech and Adi, respectively (Fig. 5b). The level of gs also declined with increasing water-stress condition and the maximum decline recorded severe-water stress was 61.76, 60.00 and 68.66% in cultivars Brukitu, Tegegnech and Adi, respectively (Fig. 5c). The E was significantly greater in Tegegnech than in other cultivars. However, it decreased significantly under water-stress condition. The reduction was 73.75, 57.50 and 74.03% at severe-water stress condition, in Brukitu, Tegegnech and Adi, respectively. Accordingly, in

Table 5: Effects of water-stress treatments on the various physiological attributes in selected cultivars of *Pisum sativum*

		Water- stress treatments			
Physiological attributes	Cultivars	T1	T2	T3	T4
Relative water content (%)	Brukitu	69.440±4.73^{b}	62.010±4.49^{c}	53.620±4.74^{d}	47.420±5.42^{e}
	Tegegnech	76.460±4.82^{a}	70.950±5.78^{a}	65.080±5.27^{bc}	50.340±4.84^{d}
	Adi	67.320±4.39^{b}	60.440±5.48^{c}	50.890±5.12^{d}	42.240±4.75^{e}
Maximum quantum yield of PS II efficiency (Fv/Fm)	Brukitu	0.795±0.05^{b}	0.772±0.04^{b}	0.765±0.02^{c}	0.708±0.03^{d}
	Tegegnech	0.813±0.04^{a}	0.808±0.05^{a}	0.786±0.04^{b}	0.763±0.051^{c}
	Adi	0.793±0.03^{b}	0.769±0.05^{b}	0.747±0.03^{c}	0.697±0.04^{d}
Photosynthetic rate (μ mol CO_2 m^{-2} sec^{-1})	Brukitu	4.270±0.23^{b}	4.210±0.22^{b}	3.650±0.21^{d}	2.020±0.21^{e}
	Tegegnech	5.850±0.21^{a}	5.230±0.27^{a}	3.990±0.27^{bc}	2.890±0.28^{d}
	Adi	4.220±0.25^{b}	4.110±0.19^{c}	3.440±0.24^{d}	1.900±0.27^{e}
Stomata conductance (mol m^{-2} sec^{-1})	Brukitu	0.068±0.031^{a}	0.064±0.03^{b}	0.037±0.03^{c}	0.026±0.03^{e}
	Tegegnech	0.070±0.020^{a}	0.068±0.02^{a}	0.040±0.03^{c}	0.028±0.04^{e}
	Adi	0.067±0.024^{a}	0.063±0.03^{b}	0.033±0.02^{d}	0.021±0.02^{f}
Transpiration rate (m mol m^{-2} sec^{-1})	Brukitu	1.600±0.11^{a}	0.970±0.12^{d}	0.670±0.18^{e}	0.420±0.17^{f}
	Tegegnech	1.600±0.12^{a}	1.220±0.13^{c}	0.870±0.16^{d}	0.680±0.16^{e}
	Adi	1.540±0.11^{b}	0.880±0.14^{d}	0.580±0.19^{e}	0.400±0.13^{f}

T1: Control plant and uniformly irrigated with tap water (400 mL kg^{-1} soil) at 3 day intervals to maintain 100% FC, T2: Irrigated at 6 day intervals (slight-water stress), T3: Irrigated 9 day intervals (mild-water stress) and T4: Irrigated at 12 day intervals (severe-water stress). Means within a column followed by the same letters are not significantly different according to LSD test ($p<0.05$)

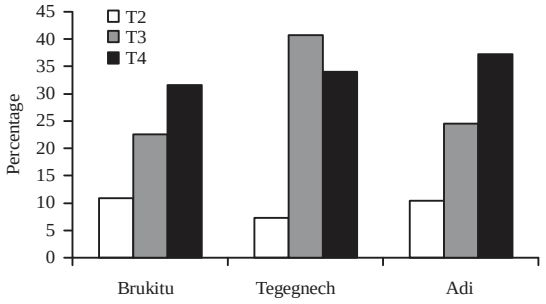

Fig. 4: Percent variation from the control, as observed under slight-water stress (T2), mild-water stress (T3) and severe-water stress (T4) conditions for relative water content in Brukitu, Tegegnech and Adi cultivars of *Pisum sativum*

terms of percent variation and the mean data value, cv Tegegnech was least effected under slight to severe-water stress condition, therefore demonstrating a superiority over Brukitu and Adi for studied physiological attributes (Table 5 and Fig. 5a-d).

DISCUSSION

The responses of plants to water stress depend on the species and genotype/cultivars, the length and severity of water stress and the stage of development (Nayyar and Gupta, 2006; Husen, 2010; Loutfy *et al*., 2012; Ghane *et al*., 2012; Husen *et al*., 2014). In the present study, severe-water stress condition caused the maximum decline on plant height, basal diameter increment, number of leaves, number of branches, leaf area, leaf width and leaf length expansion while cultivar Tegegnech was less sensitive than the others. Water stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth (Morgan, 1984; Jaleel *et al*., 2008). In addition, the shoot and root growth inhibition is a common response under water stress and plant-growth rate is one of the most important agricultural indices of water-stress tolerance (Jaleel *et al*., 2009). The molecular mechanism of plant response under water stress is almost similar to plant responses to salinity stress. In all these stressful conditions, water availability to plant cells is restricted. Therefore, as the first response, the cells try to save the available water by avoiding active growth. In the present study, water stress also affects biomass of different plant organs. The significant reduction in size and number of leaves is also linked to a reduction in biomass accumulation. The reduction in total leaf area due to water stress may be linked to the decrease in the leaf turgor. In terms of biomass accumulation, Tegegnech was greater in comparison to cultivar Brukitu and Adi. In general, reduction of biomass is positively correlated with increase in water-stress duration/condition. This might involve suppression of cell expansion per cell growth due to low turgor pressure (Jaleel *et al*., 2008). Decreased leaf area reduces the ultimate yield due to limited photosynthesis (Ge *et al*., 2012). Under a short-term water-deficit condition, significant losses in terms of plant growth and final yield have been reported earlier also (Anjum *et al*., 2008; Husen, 2010; Husen *et al*., 2014). Reduction in dry mass under water stress could be due to

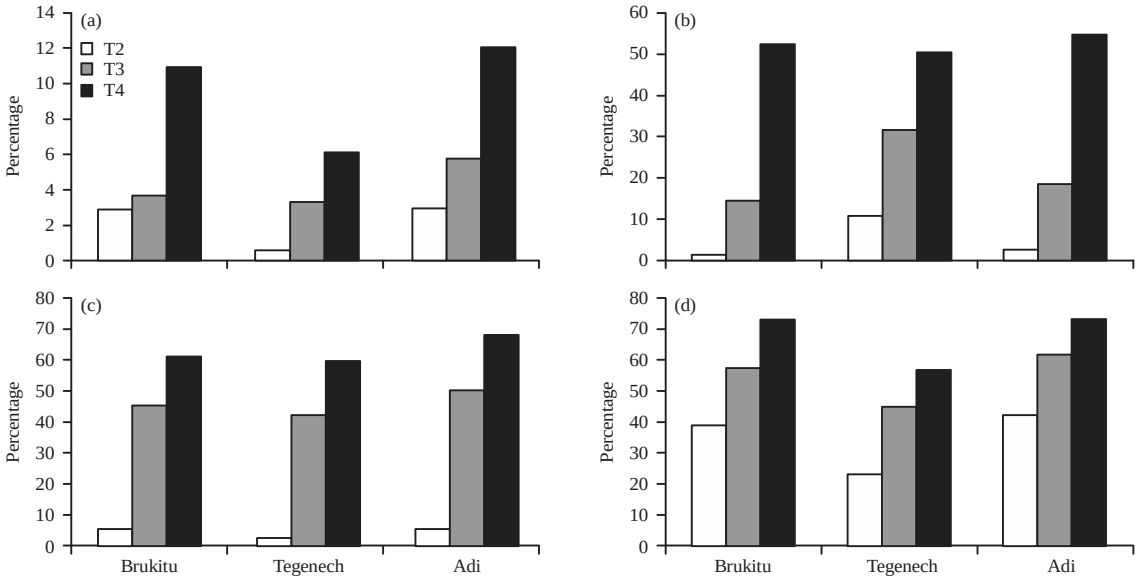

Fig. 5(a-d): Percent variation from the control, as observed under slight-water stress (T2), mild-water stress (T3) and severe-water stress (T4) conditions for, (a) Chlorophyll fluorescence, (b) Net photosynthetic rate, (c) Stomatal conductance and (d) Transpiration rate in Brukitu, Tegegnech and Adi cultivars of *Pisum sativum*

limited leaf expansion. Stomatal conductance, leading to reduced carbon assimilation per unit leaf area, ultimately results in low biomass production (Medrano *et al*., 2002).

Like some previous reports on *Brassica carinata* (Husen *et al*., 2014), *Guizotia abyssinica* (Ghane *et al*., 2012) *Catharanthus roseus* (Jaleel *et al*., 2008), *Gossypium hirsutum* (Massacci *et al*., 2008), *Tectona grandis* (Husen, 2010), *Triticum aestivum* and *Zea mays* (Nayyar and Gupta, 2006), the present investigation also revealed that the contents of photosynthetic pigments declined with increase in slight to severe-water stress condition. Thus, the decrease in chlorophyll content was more pronounced at the severe water stress condition. Chlorophyll content has a direct bearing on growth and productivity of the plant. It has been reported that chlorophyll reduction can take place as a result of increase in degradation as well as decrease in the synthesis of chlorophyll due to stress-induced metabolic imbalance (Ashraf *et al*., 1994; Dos Santos *et al*., 2004; Srivastava *et al*., 2010). It has also been reported that the inhibitory effects of decreased water content on leaf development, reduced light interception and stomatal conductance leading to a decrease in carbon assimilation (Medrano *et al*., 2002; Fariduddin *et al*., 2009) might have contributed to decreased chlorophyll content, which ultimately affect the transfer of photosynthetic assimilates from source to sink or malfunction of the photosystem (Woodward and Bennett, 2005; Bashir *et al*., 2015). In the present study, *P. sativum* cultivars were not uniform in terms of pigment concentration and other attributes. Among the cultivars, the highest chlorophyll content was recorded in Tegegnech in comparison to Brukitu and Adi cultivars. This could well be a case of hormesis (Aref *et al*., 2015).

The decline of the photochemical efficiency of PSII (Fv/Fm) under water stress, as observed in this study also, suggests that water stress affected some process related to the photochemistry of photosynthesis. The reduction in the Fv/Fm value under stressful condition, duly correlated with a decrease in different photosynthetic parameters and biomass production, is being used as an indicator for determining the seedling-stock quality (Husen, 2009, 2013; Kalaji *et al*., 2011; Husen *et al*., 2014, 2016; Getnet *et al*., 2015; Oukarroum *et al*., 2015). Tegegnech had a higher Fv/Fm value than the other cultivars. It is important to note, however, the severe-water stress condition caused a significant difference from the control, slight-water stress plant population did not, thus suggesting an insignificant impact at low water stress level on the PSII-reaction centers. In the mild to severe-water stress condition, the effects of stress upon the photochemical system were exhibited by significant decreases in the maximum quantum yield of PS II accompanied by increases in the levels of minimum fluorescence. These variations possibly reflect a disorder in PS II (Osmond, 1994). Hence, possibly, in an elevated water stress in *P. sativum* cultivars, the ability of protective mechanisms was surpassed. The decline in net photosynthetic rate, stomatal conductance and transpiration rate values was varied under mild to severe-water stress

condition in all the cultivars. A similar pattern was observed by many workers (Reddy *et al*., 2004; Galmes *et al*., 2007; Husen, 2010; Pan *et al*., 2011; Ashraf and Harris, 2013; Husen *et al*., 2014). However, cultivar Tegegnech was relatively superior in terms of net photosynthetic rate in comparison to Brukitu and Adi cultivars. It has been reported that water-deficit stress induces oxidative stress because of the inhibition of photosynthetic activity due to the imbalance between light capture and its utilization. In addition, water stress can also directly influence the rates of photosynthesis due to the decreased CO_2 availability resulting from stomatal closure (Flexas *et al*., 2006; Chaves *et al*., 2009) and/or from changes in photosynthetic metabolism (Lawlor, 2002).

From the present study, it can be concluded that water stress significantly decreases the vegetative growth, biomass accumulation, water status, photosynthetic pigments, gaseous exchange and photosynthetic efficiency in the *P. sativum* cultivars. The three cultivars tested differ in their sensitivity to water-stress level. Cultivar Tegegnech was more tolerant to water stress-induced damage than Brukitu and Adi cultivars. Thus, Tegegnech can be used further to identify and manipulate the genes controlling these traits in breeding programmes.

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